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Pre-Columbian treponemal infection in Denmark?- a paleopathological and archaeometric approach

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Abstract

The aim of this paper is to investigate five new cases of possible treponematosis in early medieval Denmark. A total of 1018 skeletons from Danish and present day German cemeteries have been examined, and five candidates of pre-Columbian individuals have been selected from three Danish burial sites on Funen, Jutland and Zealand. The five individuals with a possible infection with treponematoses have been analysed anthropological and chemically. Two of the skeletons exhibited cranial lesions, *i.e.* serpiginous and focal superficial cavitation. The other three only displayed periosteal reactions of varying degrees on the postcranial skeleton. Computed tomography scans showed focal obliteration of the periosteum on the long bones as well as on the affected skulls. Radiocarbon dates and stable isotope analyses indicate that three of the four analysed skeletons predate AD 1493. High levels of mercury in three of the individuals suggest that medical treatment with Hg-containing medicine took place.
Considering the climate and geography at the sites, venereal syphilis might be the treponemal disease causing the pathologies on the skeletal remains. However, the historical background, the bone lesions and their prevalence point to the presence of a less aggressive, maybe non-venereal, form of treponematosis. Consequently, the hypothesis that pre-Columbian venereal syphilis existed among the analysed skeletal material is rejected with the help of various archaeometric analyses.

Keywords: Syphilis, Treponematoses, Osteology, Paleopathology, Mercury, Refshale, St. Albani, Tirup

Introduction

Recently Ortner stated that "*the presence of syphilis in the Old World before 1500 A.D. remains a very contentious issue…*" [1]. For centuries researchers have discussed three main theories about the origin of syphilis. Especially one of the hypothesis, namely the existence of a pre-Columbian *Treponema pallidum pallidum* as the causative agent of venereal syphilis in the Old World has been and still is an on-going matter of dispute cf. [2-4]. The rash spread of the first sexually transmitted disease (STD) in Europe within a few years after AD 1493 has given cause for several discussions about its origin. Lack of evidence in the historical sources prior to this date suggests that venereal syphilis was completely unknown among the European population at that time. In accordance with the other European historical sources venereal syphilis does not seem to have reached Denmark prior to 1495 [5-7]. However, a precise year for the onset of the new disease cannot be given, especially because none of the sources were written prior to 1501 [8,9]. Meyer *et al.* [10] explained the sudden numerous mentioning of a "*new disease*" in the literature with the invention of the printing press by Gutenberg in the middle of the 15th century. They also mention the possibility of misdiagnoses with other known diseases prior to the 16th century, which seems quite plausible since even today syphilis is called "*the great imitator*" [11], as its clinical manifestations in the human body are mimicking several other diseases [12]. The research of Harper and colleagues [13] disproved most of the alleged pre-Columbian dates of single findings of syphilis from the Old World and outlined the uncertain diagnosis of those dated before AD 1493. Only the Anglo-Saxon case

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described by Cole and Waldron [14] seems challenging and is according to Ortner [1] unlikely to be associated with a disease other than syphilis. There are however two possible finds of non-venereal treponemal disease from pre-Columbian England and Germany. Kuhnen *et al.* [15] present a case of possible treponarid in the skeleton of a knight from early 12th century Germany; and Stirland [16] describes four adult individuals with treponemal bone changes, which Rothschild [17] determined as classical lesions of yaws. Radiocarbon dates of calAD 1025–1290 supported a pre-Columbian time [13].

Previous research [18-20] provided the foundation for this study, which is part of the first extensive search for pre-Columbian treponematoses in Denmark and present-day Northern Germany. A total of 1018 individuals have been examined until now. The present paper focuses on five putative pre-Columbian cases from medieval Denmark with bone changes attributable to treponemal infection. We have used radiocarbon dating, mercury analysis as well as radiological imaging to support the osteological examination. The tentative conclusion is that venereal syphilis did not occur in Denmark before the great epidemic, but it cannot be excluded that another treponemal strain was present in Denmark prior to 1493.

Material and methods

Five skeletons have been selected out of 327 that were examined from the three cemeteries St. Albani (Funen), Refshale (Lolland) and Tirup (Eastern Jutland). The cemeteries were chosen on the criteria that they have archaeological dates including 1493 and because of their location (rural as well as urban). The objective was to look for significant differences in treponemal lesion prevalence. The burial site of St. Albani Church in Odense represented an urban population and encompassed a total number of 172 graves. The skeletal remains are generally badly preserved, which is due to several replacements caused by adding burials through a period of 400 to 500 years [21]. The archaeological date of the skeletal remains from St. Albani churchyard is between AD 1050 and 1530 [21]. According to Becher and Gregersen [22] it is likely that part of the burials were connected to the St. Knud's Church in Odense, which was combined with those of St. Albani after the demolition of the parish church. 114 individuals were examined from these two sites; three of them will be presented in this study. A total of 134 skeletons were examined from Refshale graveyard, which is situated in the Maribo district on Lolland, a major island southwest of Zealand. Refshale cemetery was in use from AD 1100–1350 [23]. Six individuals (graves 20, 34, 130, 161, 184, and 191) are of special interest, but only the skeletal material from grave 130 will be discussed here since it shows lesions on multiple bone elements.

The third cemetery included is that of Tirup which is located close to the medieval town of Horsens in Jutland and appears to have been in use from AD 1150 to 1350 [23]. The cemetery is said to be representative for the rural Medieval Denmark [24]. Only one out of 79 examined individuals were selected for this study. A complete statistical analysis of the churchyards is available in the Master thesis of Schwarz from 2009 [25].

Age and sex estimation

The age estimations are based on published standards utilizing metamorphosis of the auricular surface [26], the pubic symphysis [27] and that of the os pubis [28]. Ageing of sub-adults was basically done by means of the dental development and eruption [29,30], as well as the epiphyseal fusion [31]. In addition to subjective aging the so-called transition analysis designed by Boldsen *et al.* [32] was used if applicable. The sexual dimorphism of certain characteristics in shape and size on the skeleton was evaluated in order to estimate the sex of an individual. The most distinct changes can be observed on the os coxae (greater sciatic notch and subpubic concavity). Further markers can be found on the cranium, *i.e.* shape of the glabella, the morphology of the supraorbital margin, size of the mastoid process, the relief of the superior nuchal line and the shape of the gonial angle as well as the anterior mandible [33]. Considering all the observable morphological changes, a subjective age and sex determination has been given.

Errors in age and sex estimation

Age diagnosis of adult individuals is far more problematic than that of sub-adult individuals. The human skeleton is changing a lot during childhood, *i.e.* bone growth, ossification of epiphyses and of course the dental development. All of those are excellent age markers, which allow age estimation with an accuracy of 1–2 years [34]. The adult skeleton on the contrary only changes a little during life, so the age is usually given with an inaccuracy of 10–20 years. The greatest error is usually in individuals older than the cases described here. In addition diet, disease, physical activity as well as cultural differences can cause variations of the above-mentioned criteria for age and sex estimation. This variability can lead to some misinterpretation of the actual age-at-death and sex of an individual.

Paleopathology

Pathological conditions for syphilis, leprosy, FOS (Focal Osteolytic Syndrome) and tuberculosis were scored after a manual created at ADBOU [35] including new scores adapted from [36,37] and Schwarz (unpublished observations). Three out of four human treponematoses are leaving traces in the skeleton and it is therefore possible to diagnose them in the archaeological skeletal material [3,38,39]. Those three are venereal syphilis (*Treponema pallidum pallidum*), endemic syphilis (*T. pall. endemicum*), and yaws (*T. pertenue*). The fourth infection, pinta, caused by *T. carateum* does not affect the bones. Based on what is known about the treponematoses today, they have geographical, sociocultural and climatic preferences [38]. But as already mentioned by Cole and Waldron [14] those preferences need not necessarily be the same during the European Middle Ages. Especially the prevailing hygienic conditions could have favoured a transmission via skin-to-skin contact or by *e.g.* sharing the same eating utensils as in non-venereal treponemal diseases. It should also be stressed that the clinical manifestations of the treponematoses might have changed over time [40], which implies that the paleopathological evidence is evaluated on the basis of what the bone lesions look like today.

The bone lesions of the three treponematoses affecting the skeleton are not distinguishable from each other. Descriptions of the lesions are mainly based on Hackett [2] as well as on Aufderheide and Rodriguez-Martin [38]. Lesion distribution as well as their shape, size, number and location within the bone were considered for differential diagnosis [38,41]. Also the on-trial-criteria established by Hackett [2] were considered in order to include possible early stages of treponemal disease.

The macroscopical examination was supported by computed aided X-ray tomography (CT), which was carried out at the Clinic of Radiological Diagnostic at the University Hospital of Schleswig Holstein in Kiel (using a SOMATOM Sensation 64 manufactured by Siemens) and at the Institute of Forensic Medicine of the Odense University Hospital (a SOMATOM Spirit, Siemens). The CT-method provides better imaging of pathological lesions in bones than normal X-rays [41]. Radiological imaging can assist in establishing a differential diagnosis of bone lesions. However, the visible lesions are not necessarily specific, but can help to exclude other diseases, *e.g.* bone tumours. In addition the visible lesion patterns have to be distinguished from normal anatomical variants [41]. According to Resnick and Kransdorf [42], syphilis and the other treponematoses can appear as periostitis (inflammation of the periosteum), osteitis (inflammation of the cortex) and osteomyelitis (inflammation of the bone and bone marrow). It needs to be stressed here that neither X-ray nor CT-scanning can distinguish between the different causative agents of treponemal infection. CT-scans were performed on all five Danish individuals with possible treponemal lesions.

Dating and stable isotope analyses

Archaeological dating

It has been suggested that burials from the Danish medieval cemeteries can be dated by means of the predominant arm position [23,43]. Kieffer-Olsen [23] observed four different arm positions in Southern Scandinavian during the Middle Ages: arm position A (AD 1100–1250), arm position B (AD 1250–1350), arm position C (AD 1350–1536) and arm position D (from AD 1536 onwards). This method is recognized as a reliable dating method for the Middle Ages in Denmark by several authors [22,44], but the arm position methodology has not been substantiated by scientific dating methods such as radiocarbon dating, dendrochronology or thermoluminescence.

Radiocarbon dating and stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$)

Additional to the archaeological dating the radiocarbon dating method was used in order to meet the standards for analysing putative pre-Columbian material [13] and to test and augment the results of the arm position chronology. The measurements were conducted at the Leibniz Laboratory for Radiometric Dating and Isotope Research in Kiel, Germany. The conventional ages were calibrated using the computer program OxCal v4.1.7 [45] and the INTCAL09 calibration curves [46].

Skeletal remains from individuals consuming a diet rich in marine proteins are subject to a radiocarbon reservoir effect, which makes dates appear older than they actually are. The effect can be corrected for by subtracting a reservoir correction, which can be calculated from the stable isotope ratio, $\delta^{13}C$. The precise calculation of the reservoir correction can be done in various ways. The following formula is from Rasmussen *et al.* [47] and was calculated on the basis of 25 years of measurements done at the Copenhagen Radiocarbon Laboratory:

$$K_{reservoir} = 410 * \left(19.8 + \delta^{13}C_{Sample}\right)/8.9\ {}^{14}C\text{–years}$$

Another reservoir effect may arise from the intake of freshwater fish protein. The $\delta^{15}N$ values of human bone collagen can be used to distinguish between a normal terrestrial diet and a freshwater fish diet. Nitrogen isotope values are enriched by 3–4‰ in the bone of the consumer when dieting on a food source one trophic level higher [48]. Even if the $\delta^{15}N$ ratio is known, it is at the moment not possible to calculate a reliable freshwater reservoir effect. However, from the $\delta^{15}N$ it is possible to judge *if* the individual has been subjected to a freshwater reservoir effect at all. In this study the measurement of the stable isotopes $\delta^{15}N$ was carried out with isotope-ratio mass spectrometry (IRMS) by Dr. Ulrich Struck at the Museum für Naturkunde, Leibniz Institute for Research on Evolution and Biodiversity in Berlin.

Mercury analysis

Mercury was used in medical treatment already in the early medieval times [49], but got more popular during the late medieval times as an alleged cure against syphilis. Mercury poisoning almost never leads to pathological bone lesions [50], but can be detected in bones as well as in teeth and hair samples by cold vapour atomic absorption spectroscopy (CV-AAS). Normal Hg-levels in human bones from medieval Denmark were 10–100 ng g^{-1} [47]. Higher levels of Hg in the bones could point to a treatment with an Hg-containing medicine.

Great care was taken when drilling samples from the bones in order to avoid contamination. Lab coats, plastic gloves, hairnets and facemasks were used throughout the sampling procedure. Two layers of Al-foil covered a large sheet of white paper, and the bone was placed on top. A conical drill mounted on a Dremel MultiPro® electric drill was rinsed in MilliQ water and heated to dryness in an ethanol flame before drilling in order to rid the surface of any Hg present on the drill. A small area of bone surface was drilled off and discarded together with the gloves and the top layer of Al-foil. The drill was cleaned in MilliQ® water and heated once again in the ethanol flame. A new sheet of Al-foil was added and new gloves were used. The bone sample was drilled at the same place without touching the remaining bone surface surrounding the decontaminated spot. Approximately 50 mg bone granulate was transferred to a pre-cleaned glass vial and sealed. After weighing ca. 20 mg of sample was transferred to a centrifuge tube. The bone material was dissolved in 4 mL concentrated analytical grade HNO_3 (69%) and 2 mL concentrated analytical grade H_2O_2 (30%). Afterwards the tubes were placed on a shaker for 3–4 hours, following which 0.67 mL concentrated analytical grade HCl (37%) was added in order to remove any superfluous H_2O_2. The samples were left overnight on the shaker with the caps loosely fitted. 3 mL of the solution was diluted with 16 mL of MilliQ water. 1 mL of 5 ppm $KMnO_4$ was added in order to keep the Hg in an oxidized state. The samples were placed on the shaker for at least two hours to allow the reactions to run to completion. Standard solutions in the concentration range 25 – 200 ppb were prepared using mercury nitrate in diluted HNO_3 (1.5%). The Hg was measured by cold vapour atomic absorption spectroscopy on a Flow Injection Mercury System 400 manufactured by PerkinElmer. The Hg was released as vapour in the FIMS-400 by adding $NaBH_2$ (0.2%) in 0.05% NaOH. Samples, blanks and standard solutions were measured as well. The analyses were then run in triplicate in an alternating sequence, with a blank between each of the samples.

Results

Macroscopical examination, dating and CT-scanning

The macroscopical examination of 375 skeletons from three Danish cemeteries revealed five skeletons of special interest because of their treponemal-like lesions and their possible pre-Columbian date (Table 1). It should be noted that only some of the individuals meet the diagnostic criteria, while others show multiple of the on-trial lesions for treponematoses (Hackett [2]).

St. Albani, Skeleton 89

Only the postcranial skeleton is present, which made ageing and sexing of the individual difficult. The individual is most likely an adult female. No arm position was observed, but radiocarbon dating gave a calibrated age interval of AD 1435–1456 (1 sigma), which is an accurate radiocarbon date. The surfaces of the tibiae appeared very uneven and rugose; several spots with bone deposits are merging into the surrounding bone with diffuse borders. Both tibiae are displaying nodes with striation (Hackett p. 78 [2]) on the medial side of the diaphysis. One of the nodes on the diaphysis of the right tibia shows superficial cavitation (*diagnostic criterion*, Hackett [2]). The tibiae shafts are slightly bowed. No concomitant pathology was observed on the remaining skeletal elements. A CT-scan of the right tibia revealed an osteolytic lesion in the new bone on the anterior part and very low density of the original bone (Figure 1).

St. Albani, Skeleton 90

Compared to the overall condition of the skeletal remains from the St. Albani churchyard the skeleton of burial 90 is in a good state of preservation. The skeleton was aged using transition analysis as established by Boldsen *et al.* [32]. The lowest possible age was 15 years while the upper end estimate was 22.7 years at a level of 95% confidence. The epiphyseal fusion [31] and dental development [29] placed the individual in the late-juvenile to early-adult category in compliance with the transition analysis based age estimation. The pelvic morphology and a general robust appearance of the skeletal elements suggest that it is a male. The observed arm position dates the individual to between AD 1250–1400 [23], but the calibrated radiocarbon age is AD 1443–1466 at 1 sigma. Skeleton 90 has multiple lesions on several skeletal elements. The bone lesions on the right parietal are focal superficial cavitation and clustered pits [2]. CT-scanning show increased density of the diploë and thickening of the tables. The outer table is partially interrupted by round notches, which leads to necrotic damage in the diploë (Figure 2). The focal radiolucent areas indicate an infection with a treponemal disease. The proximal part of the right femur diaphysis exhibits high radiodensity suggesting new bone growth of the

Table 1 Summary of the archaeometric analyses

Sample	Age group	Sex	Location	Lesion description (after Hackett, 1976)	Calibrated radiocarbon date AD(1σ)	Hg ± 1σ (ngg^{-1})
St. Albani-89	adult	probably female	urban	Nodes with striation, superficial cavitation	1435-1456	374 ± 8
St. Albani-90	early adult	male	urban	Superficial cavitation and clustered pitting	1443-1466	388 ± 7
St. Albani-94	mid-adult	male	urban	Rugose nodes and expansions	1453-1494	390 ± 6
					1602-1614	
Refshale-130	mid-adult	female	rural	Finely striate and coarsely pitted expansions	1160-1212	22 ± 3
Tirup-292	early-adult	female	rural	Serpiginous cavitation	n.d.	25 ± 3

cortex. An osteolytic lesion of around 3.5 cm in diameter is placed in the trabeculae of the trochanter major. Further changes are seen on the humerus as periosteal reactions and cortical thickening mainly in the distal end. These are visible as radiolucent and sclerotic areas on the CT-scanning. The scan of the ischium showed diffuse lytic structures, which were visible as clustered pitting on the surface of the bone. Changes on the tibia bone were observed: a thickened cortex and diffuse changes on the anterior surface continuing into the substantia compacta.

St. Albani, Skeleton 94

Skeletal indicators suggest that this individual is a male. The changes on the pubic symphysis as well as on the auricular surface are consistent with an individual who was 35–45 years old when he died. Arm position B places the burial in between AD 1250–1400, but radiocarbon age indicates a younger age, calAD 1453–1494 (1 sigma), but could also be as late as calAD 1602–1614. Bone changes of the right tibia fit Hackett's on-trial criteria of rugose nodes and expansions (Hackett p.106 [2]). CT scanning revealed several osteolytic foci and the destruction of the cortical structure plus bone expansions (Figure 3).

Figure 1 CT scans Skeleton from 89St. Albani, Odense. 3D surface-rendered image of the right femur (right) and tibia (left). The scan displays periosteal reactions (blue arrows) on the anteriorly bowed tibial cortex (sabre shin; edited with OsiriX v.5.5.2 [51]).

Refshale, Skeleton 130

Skeleton 130 is a completely preserved adult female. Transition analysis suggests an age between 24.7 to 46.4 years with a maximum likelihood of 32.9 years. The skeleton is radiocarbon dated to calAD 1160–1212 at ± 1 sigma. Changes observed on nearly all the long bones indicate a systemic disease. Left radius shows coarse striations on the lateral distal side as well as coarsely pitted and moderate rugose new bone on the medial distal end of the diaphysis. The right radius is giving the same picture as the left one but with small, round and oval shaped depressions. Distal ends of both humeri display clustered pitting. The femora have expanded diaphysis with rippling of the surface and coarse pitting. Both fibulae are thickened and there are areas with new bone layers in the distal end of the bones. The posterior surface of the tibiae shows multiple channelled impressions of blood vessels. On the anterior and medial side periostitis and linear striations are dominating most of the diaphysis. The shaft is slightly bowed anteriorly. Small areas of slightly higher radiodensity on both the tibia and the femur could also be results of a normal variation (Figure 4).

Tirup, Skeleton 292

The individual from burial 292 from Tirup cemetery was most likely a female around 20–24 years. The woman was buried with arm position A (AD 1100–1250), which is in accordance with the period of use of the graveyard (AD 1100–1350). Besides postmortal damage, the skull shows serpiginous cavitation on the frontal bone (Figure 5). These changes are continuing into the right orbit. Remodelling is seen of the nasal aperture as well as the inferior nasal conchae. None of the other preserved skeletal elements, *i.e.* tibiae, scapulae, ribs, ulnae or mandible displayed any lesions indicative of

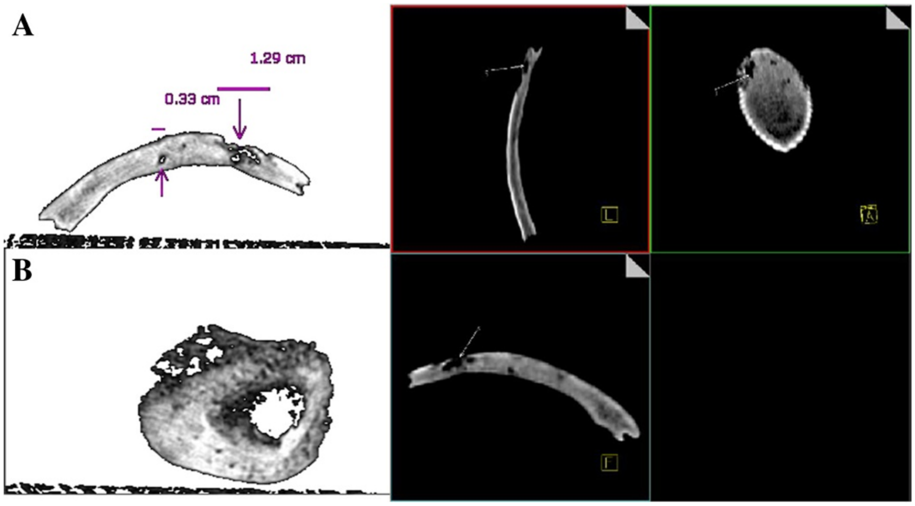

Figure 2 CT scans of skeleton 90 from St. Albani, Odense. Left: **A** Right os parietale displaying osteolytic lesions and a worm eaten appearance on the outer table, **B** right tibia with diffuse lytic changes on the anterior part. Right: Multiplanar reconstruction image of the parietal bone (edited with OsiriX v.5.5.2).

treponematoses. The changes on the frontal as well as the nasal bones are regarded as diagnostic for treponemal disease (Hackett p. 45 [2]), but can also occur in bejel and yaws [52]. These changes are visible as translucent areas on the CT-scans (Figure 6).

Mercury analysis

The Hg analyses of four femurs and one tibia bone samples showed high levels of Hg in three of the individuals. These three individuals are all are from the St. Albani churchyard in Odense. Samples taken from the femur bones of the individuals in grave 89, 90 and 94 revealed Hg concentrations of 374 ± 8 ng g^{-1}, 388 ± 7 ng g^{-1} and 390 ± 6 ng g^{-1} respectively. All three individuals have severe bone lesions that could be caused by treponemal bacteria, and they have apparently been treated with Hg-containing medicine. The individuals from the Refshale and Tirup cemeteries had Hg concentrations as low as 22 ± 3 and 25 ± 3 ng g^{-1} (respectively), which imply that these individuals were not exposed to high amounts of Hg during their life, but exhibit Hg concentrations typical for the non-exposed individuals who are normally showing Hg concentrations between 10 and 100 ng g^{-1} [47].

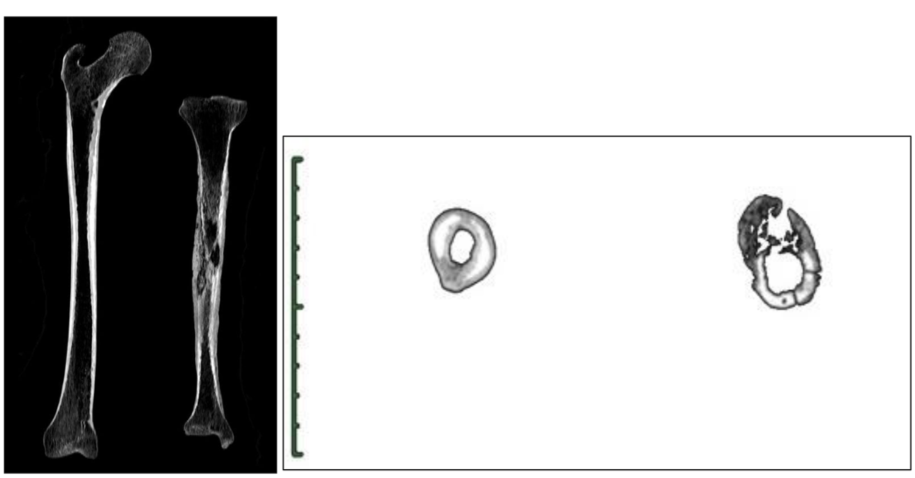

Figure 3 CT-scans of the right tibia and femur of individual 94 from St. Albani, Odense. Left: On the right side is the tibia with thickened diaphysis and several osteolytic lesions in the cortical bone. Right: The left side shows the femur with parts of high radiodensity, indicating an inflammatory process. The tibia on the right side shows intensive osteolysis of the compact bone on the anterior part and increased signal intensity in the remaining compact bone, consistent with inflammatory involvement (edited with OsiriX v.5.5.2).

Figure 4 CT-Scans of skeleton 130 from Refshale, Lolland-Falster. Left: Picture of the tibia bones with linear striations on the medial side and thickened diaphyses. Right: CT scan of the right tibia and femur. Left side showing the tibia with osteolytic changes within the cortex; right side showing the femur with increased radiodensity; both are non-specific changes (edited with OsiriX v.5.5.2).

Discussion

The application of different methods in paleopathological studies does not necessarily prove or disprove a hypothesis, but it can help to build up strong evidence for or against it. In the case of possible pre-Columbian treponemal infection in skeletal material it is unavoidable to use radiocarbon dating for more precise dating than archaeological dating and dating by arm positions. For this study Hg analysis proved a good method to support the assumption, that the individuals have been suffering of some kind of disease causing the physicians to apply an Hg-treatment. The individuals could also have been otherwise in contact with Hg, but considering the severe bone lesions, Hg-containing medicine seems to be the most likely factor causing the high concentrations observed.

The radiocarbon ages do not completely coincide with the archaeological dates of the individuals. Only in the case of burial 130 from Refshale is seen a complete accordance. The skeletal material from Tirup cemetery was not dated with the radiocarbon method, since this cemetery has been studied extensively [53-55] and has been archaeologically dated prior to 1493 with certainty. The precision of the radiocarbon dates of medieval samples are often better than ± 25 to 30 years for the 1 sigma statistical uncertainty of the calibrated age interval [56]. However, if reservoir effects are present they can introduce an offset and an added uncertainty. Radiocarbon dating of skeletal remains from coastal regions can be affected by the marine reservoir effect, which makes the dates appear older than they really are. The marine

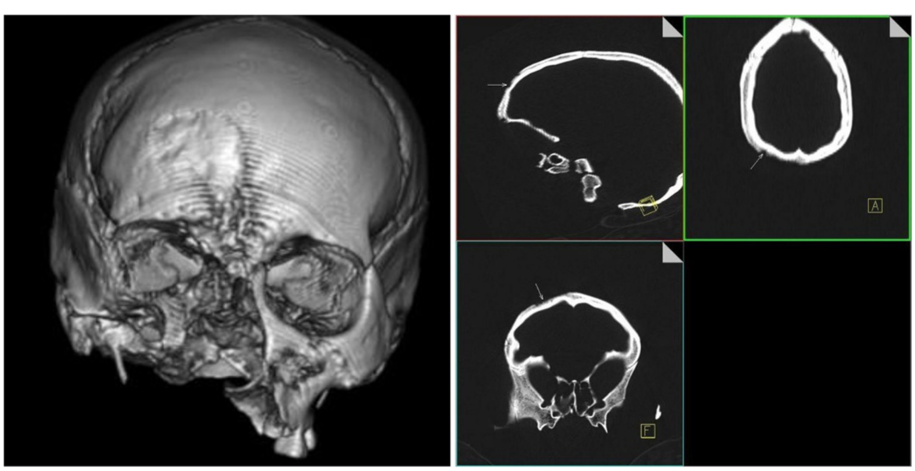

Figure 5 CT-scan of the cranium of skeleton 292 from Tirup. Left: frontal photograph of the serpeginious lesions in the orbit (blue arrow) and on the frontal bone (red arrow). Right (upper): photograph of the right lateral side of the frontal bone. Confluent clustered pitting (magenta arrow) close to the coronal suture and almost round lytic lesion above the sphenoid bone. Right (lower): transverse view of the serpeginious lytic lesion on the upper margin of the orbit (blue arrow).

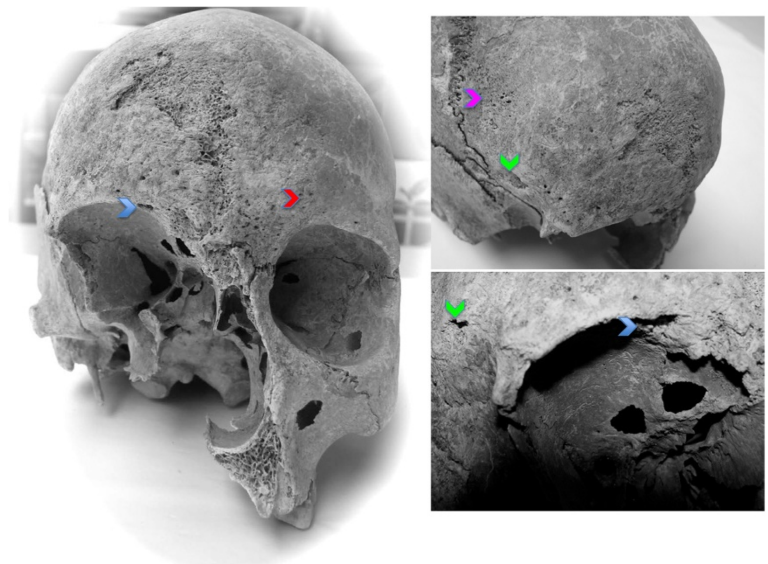

Figure 6 CT-scan of the cranium of skeleton 292 from Tirup cemetery. Left: 3D volume-rendered image of the skull (frontal view) showing serpiginious lesions on the right frontal bone. Right: 3D multiplanar reconstruction of the skull. The osteolytic lesions are marked with white arrows (edited with OsiriX v.5.5.2).

reservoir age for open waters in the North Atlantic area and the open inner Danish waters is around 410 years, which should be kept in mind when interpreting bone material of humans who derived most of their diet from the sea. The percentage of marine carbon is estimated as follows: burial 90 from the St. Albani: 1%, St. Albani 94: 11%, St. Albani 89: 13% and Refshale: 13% (Table 1). These values differ substantially from *e.g.* those derived when analysing Viking and medieval settlements from Greenland [44], which showed a predominantly marine based diet from around the middle of the 13th century. The low amount of marine food in the diet of the analysed individuals in this study, therefore underlines the reliability of the radiocarbon dates what concerns the marine reservoir effect.

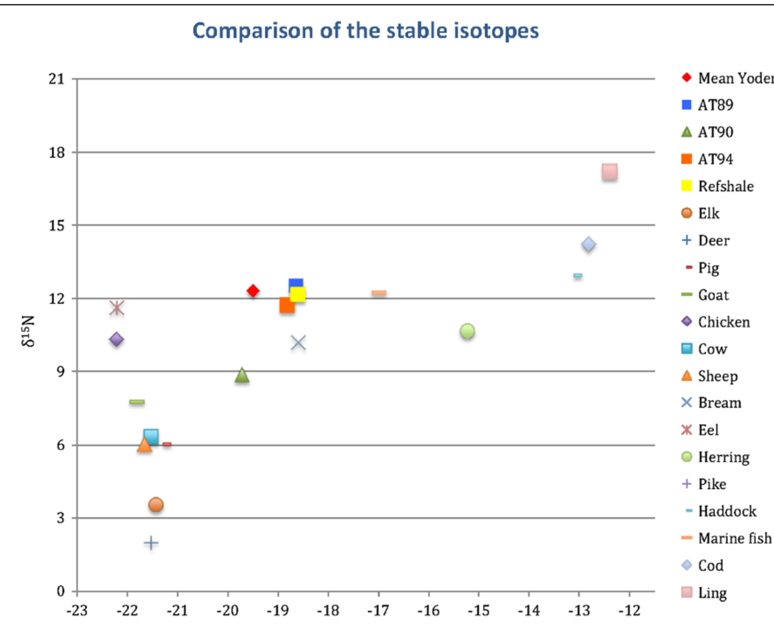

Figure 7 Stable isotope ratios $\delta^{13}C$ and $\delta^{15}N$. The samples in the present investigation compared to faunal samples published by Yoder [60].

The results of the stable isotope analyses can be compared with studies of $\delta^{13}C$ and $\delta^{15}N$ from similar sites in Denmark and Sweden (Yoder Figure 7 [57]). The C:N-ratio of all four samples is ca. 3.4 meeting the accepted range of 2.9-3.6 defined by DeNiro [58] and even the narrow range of 3.1 to 3.5 suggested by van Klinken [59] for historical samples. Three of the analysed individuals, Refshale 130, St. Albani 89 and St. Albani 94 have $\delta^{15}N$ values of 12.2‰, 12.5‰ and 11.7‰, respectively (Table 2). As these $\delta^{15}N$ values are enriched by 3–4‰ over the normal terrestrial food sources, this indicates a diet including a large proportion of freshwater fish [60]. As noted before a freshwater reservoir effects can be as high as 100 to 1000 years [61]. The radiocarbon ages of all three individuals can therefore be younger than the ages quoted here (which is without a freshwater reservoir correction) by an unknown amount. It is therefore possible that all three individuals can have died after 1493.

The $\delta^{15}N$ results for burial 90 from the St. Albani churchyard, on the other hand, has a value of 8.9‰ which points, according to Yoder (p. 89 [60]), to a more terrestrial protein rich diet, for instance meat, milk etc. (5–10‰). This is also the only individual with a marine diet as low as 1%, which means that the radiocarbon age for this individual, can be considered to be completely reliable. This fact points to an error in the archaeological date by arm position (AD 1250–1400) as compared to reliable radiocarbon date of calAD 1443–1466 at 1 sigma.

Out of the five cases studied here, two cannot be excluded to be later than AD 1493. One individual, St. Albani 90, is for sure pre-Columbian, and has therefore the full potential to be a pre-Columbian case of treponemal infection.

According to Livington [62] the species that are restricted by geographical conditions are the oldest of the treponemal diseases and both the endemic and the venereal syphilis evolved out of one of these [63]. While the non-venereal forms are transmitted either via skin-to -skin contact or other non-sexual contact, it had to find a new way of transmission. That is probably why it adapted to a venereal form to evade the better hygienic conditions and to acclimatise to a colder climate [64]. Today, only 15% of all untreated patients with venereal syphilis develop bone lesions [65] this indicates an adaptation from an aggressive to a less aggressive disease. The most prominent pathognomonic changes of syphilis are those of the *caries sicca sequence* seen on the cranial vault [2]. This condition is present in one of the skeletons from St. Albani and in the one from Tirup. Lesions consistent with treponemal disease are found on several long bones, but only those in skeleton 90 from St. Albani are indicative of treponemal infection. The observed lesions are bilateral and systemic, as is characteristic for the treponematoses. Since tuberculosis can be excluded the focal superficial cavitations can be regarded as a diagnostic criterion for treponemal infection (Hackett p. 36 [2]).

Differential diagnosis

Possible differential diagnoses for treponemal like lesions are that of infectious diseases, such as leprosy and tuberculosis, Paget's disease, fungal infections, pyogenic osteomyelitis and bone tumours. Both Refshale and Tirup have been analysed for leprous bone changes earlier [66]. Boldsen [66] describes the destructive lesions on the facial and phalangeal bones as well as hyperostosis with or without exostoses on the tibia and fibula as

Table 2 Results of the radiocarbon dating

Sample	Archaeo-logical Date[a]	Uncalibrated age(BP)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N	Estimated marine diet (%)[b]	Reservoir correction[c]	Corrected ^{14}C age[c](BP)	Radiocarbon date uncorrected for marine signature, 95% CI, (calAD)[d]	Radiocarbon date corrected for marine component, 95% CI, (calAD)[e]
Sct.Albani94	1250- 1350	425 ± 20	−18.82	11.73	3.4	11	55	380 ± 20	1434-1483	1447- 1521
										1576–1582
										1591- 1621
Sct.Albani89	n.d.	490 ± 25	−18.64	12.53	3.4	13	53	437 ± 20	1410-1446	1425- 1482
Sct.Albani90	1100- 1400	420 ± 20	−19.72	8.88	3.4	1	4	416 ± 25	1435-1488	1437- 1489
									1604-1608	1604- 1607
Refshale 130	1250- 1400	925 ± 25	−18.61	12.18	3.4	13	45	870 ± 25	1029-1165	1047- 1089
										1121–1139
										1149- 1224

[a]The archaeological date is based on a chronology of different arm positions used during the Southern Scandinavian Middle Ages [24,44].
[b]Estimation of % marine diet is based on linear interpolation with an endpoint of −10.9 ‰ (assuming a 100% marine diet) and an endpoint of −19.8‰ assuming a 100% terrestrial diet [50].
[c]The following formula was used to calculate marine reservoir correction: $K_{reservoir} = 410 * (19.8 + \delta^{13}C_{sample})/8.9$ ^{14}C – years (Rasmussen *et al.*, 2008 [47]).
[d]OxCal v4.1.7 [34]; Atmospheric data from [49].
[e]CALIB REV6.0.0 (Dataset: IntCal09, [49]).

characteristic bone changes for leprosy. Ortner [39] writes that this *"...pathological reactive bone is most severe near the ankle and diminishes in severity toward the knee..."*. None of the five skeletons described in this paper shows any lesions diagnostic of leprosy. Tuberculosis is usually affecting the spine and the inner table of the cranium [38] while hypertrophic expansion of the long bones is described as unusual (p.163-4 [38]). Hypertrophic lesions are also unlikely for a fungal infection. Paget's disease is excluded for the cases described here, because no marble-like bone thickening was present on the CT scans [38]. Changes caused by pyogenic osteomyelitis are characterized by cloacae and sequestrate, but usually do not involve multiple skeletal elements (p. 163–4 [38]). Bone tumors can be metastatic carcinoma, osteogenic sarcoma, multiple myeloma and meningioma. Changes associated with bone tumors are solitary, lytic lesions (p.93 [41]) and periosteal reactions in the radiological image.

Conclusions

In the present study five skeletons were selected amongst 1018 Medieval Danish individuals as potential candidates of pre-Columbian venereal syphilis. The documented lesions of three of them only meet the descriptions of Hackett's on-trial criteria, but two are also displaying diagnostic criteria. The results of our analyses show that those two, Tirup 292 and St. Albani 90, can be securely dated to pre-Columbian times. The limitations of the study are that the results of the archaeometric analyses applied here cannot elucidate the origin of the probable treponemal bone lesions. However, the Hg analysis is found to be a helpful tool when analysing skeletal remains from the medieval times for pathologies. In the present case it revealed that three out of the five individuals with treponemal-like bone lesions had been treated (or otherwise in contact) with Hg-containing medicine. This supports historical sources, which describe the treatment of skin diseases, especially leprosy and syphilis, with Hg in the medieval time. This study therefore demonstrates that Hg was used for treating treponemal diseases. This particular form of medical treatment continued right up to near-modern times for venereal syphilis.

The current knowledge about modern clinical manifestations of the treponemes in the human skeleton allows us to conclude that some kind of treponemal infection was present among the examined skeletal material in the present study. The Danish historical sources do not mention a syphilis epidemic before the return of Christopher Columbus to the Old World in 1493. Considering the currently available data [13] there are no significant findings of venereal syphilis in our modern understanding of that disease in pre-Columbian Europe. The aggressive progress of syphilis described in the literature [9] is by no means presented in the analysed skeletal material. The osteological manifestations rather draw a picture of a more benign affliction. The phylogenetic analysis by Harper *et al.* [67] showed that the syphilis strain, *Treponema pallidum pallidum,* has a shorter history of evolution compared to that of the non-venereal strains. It is therefore generally agreed that venereal syphilis evolved from the 16th century to modern times and seems to have been a new disease to the Danish medieval population at the end of the 15th century.

The present study together with the research of Stirland [16] and Cole and Waldron [14] demonstrates that it becomes more evident that some kind of treponemal infection was present in the Old World prior to 1493. Perhaps strontium isotope analysis could give a hint to whether those single findings are a result of migration during the Middle Ages [68].

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
SS coordinated the study and made the anthropological examinations. LS performed the chemistry laboratory work. SS and KLR drafted the manuscript. All authors read and approved the final manuscript.

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